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New Characeae (Charophyceae, Charales) report in eastern Sicily (Italy)

Ilaria Pagana[1], Alessandra Virzì[1], Manuel Andrea Zafarana[2], Giuseppina Alongi[1]

1 *Dipartimento di Scienze Biologiche, Geologiche e Ambientali dell'Università degli Studi di Catania, Via Empedocle 58, 95128 Catania, Italy* **2** *Stiftung Pro Artenvielfalt, Malzgasse 5, CH-4052 Basel, Switzerland*

Corresponding author: Ilaria Pagana (ilaria.pagana@phd.unict.it)

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Abstract

Nowadays species belonging to the green algal family Characeae are highly endangered. For this reason, many of them are protected by European directives and included in Red Lists. In Italy, however, the Red List of Characeae does not exist and the distribution of these species is often unknown, especially in Sicily wetlands. In this study, we investigated the distribution and morphology of Characeae in fresh and brackish water wetlands, both natural and artificial, located in various areas of eastern Sicily (Italy). During the survey *Chara globularis* Thuiller, *Chara vulgaris* Linnaeus, *Nitella capillaris* (Krocker) J.Groves & Bullock-Webster, and *Tolypella glomerata* (Desvaux) Leonhardi were found. This is the first record of these species in the investigated sites. The results improved the knowledge of freshwater algal biodiversity in Sicily and, above all, allowed to fill distribution gaps of the Characeae species, to apply adequate protection measures and create national Red Lists of Characeae.

Keywords

Aquatic flora, *Chara*, freshwater algae, *Nitella*, *Tolypella*, wetlands

Introduction

The family Characeae (Charales, Charophyceae), also called stoneworts, includes green algae with macroscopic thalli consisting of a succession of nodes and internodes of varying lengths. The nodes consist of two short central cells and 6 or more pericentral cells each of which gives origin to a ray so that a whorl of rays occurs at

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the level of each node; the nodes are separated from each other by internodes consisting of very long single cells (Bazzichelli and Abdelahad 2009). This family which includes different genera is arranged into two tribes: Chareae [*Chara* Linnaeus, *Lamprothamnium* J. Groves, and *Lychnothamnus* (Ruprecht) A.Braun] and Nitelleae [*Nitella* C.Agardh and *Tolypella* (A.Braun) A.Braun] (Bazzichelli and Abdelahad 2009; Guiry and Guiry 2024).

Species belonging to the Characeae live in fresh and brackish waters. They grow in a wide range of depths but mainly in shallow, still, or gently flowing waters. Characean species can live in artificial ditches, forest sumps, and temporary ponds, forming large meadows in almost all habitats where they occur (Wood and Imahori 1965; Bold and Wynne 1985; Lambert 2007).

All the Characeae species have an important ecological role in the environments in which they are present. For example, they maintain high water transparency, are able to store large quantities of carbon and nutrients, are the base of the trophic chain, and offer refuge and create habitat for many animals (Lucas 1975; Blindow et al. 2002; Schneider et al. 2015). They are considered good bioindicators (Lambert-Servien et al. 2006; Täuscher 2012; Pukacz et al. 2013): salinity, temperature, chemistry of water and light are factors that affect the distribution of these algae in the environments, and therefore, their presence or absence can provide information about several environmental factors (Gąbka et al. 2007; Boszke and Bociąg 2008).

Nowadays the species of Characeae are highly endangered. The most severe threats to their survival can be found in arid and semiarid regions due to the combination of continuous human transformation of the environment and climate changes (Romanov et al. 2022). For this reason, some species of Characeae are protected by the Habitats Directive 92/43/EC and the Water Framework Directive 2000/60/EC; moreover, in some European countries these species are protected by national legislation and included in national Red Lists (e.g. Stewart and Church 1992; Palamar-Mordvintseva and Tsarenko 2004; Blaženčić et al. 2006; Gärdenfors 2010; Auderset Joye and Schwarzer 2012; Korsch et al. 2013; Urbaniak and Gąbka 2014; Stewart and Hatton-Ellis 2020). In Italy, however, a national Red List of Characeae is not available. This is a major gap in the conservation legislation for the country, particularly because there are still many locations in Italy, especially in Sicily, where no information is available on the presence of these algae. The main information available in Sicily on the distribution of these species concerns western Sicily, particularly in the Palermo area (Formiggini 1908; Naselli-Flores and Barone 2002; Troia et al. 2018; Guarino et al. 2019; Romanov et al. 2019; Panzeca et al. 2021)

In this study, we investigated the distribution and morphology of species of Characeae in different freshwater and brackish wetlands, both natural and artificial, in eastern Sicily (Italy). The results improve our knowledge of the botanical biodiversity of these areas and fill, at least partly, gaps in the knowledge of the distribution of this species in Sicily.

Methods

Sampling areas

Sampling was conducted in the period spring/summer of 2022 and the sampling sites were chosen after a careful preliminary inspection of several areas in eastern Sicily. The selected sites hosted populations of Characeae with a visual coverage higher than 5%: Cesarò - Messina (LM), Gela - Caltanissetta (GW - A; GW - B; GW - C), Melilli - Syracuse (CU), and Troina - Enna (LA). Fig. 1 shows the sampling sites and was produced using QGIS 3.16 software; in Table 1 all the sampling sites with the coordinates and the abbreviations used are reported.

In detail, the sampling sites of Cesarò and Troina are located within the Nebrodi Regional Natural Park, in zone A and in zone B respectively. In zone A, sampling was done in one of the small natural lakes around Monte Soro at approximately 1,575 m above sea level (asl). This area is within two Natura 2000 sites: ITA030038 and ITA030043. In zone B, sampling was carried out in the artificial lake called "Ancipa – Sartori" at an altitude of approximately 947 m asl; this site falls within the Natura 2000 site ITA060005. In Gela sampling was carried out within the protected area called "Geloi Wetland" located at approximately 30 m asl. It is located in the "Piana di Gela" and falls within the Natura 2000 site ITA050012. In this area, which

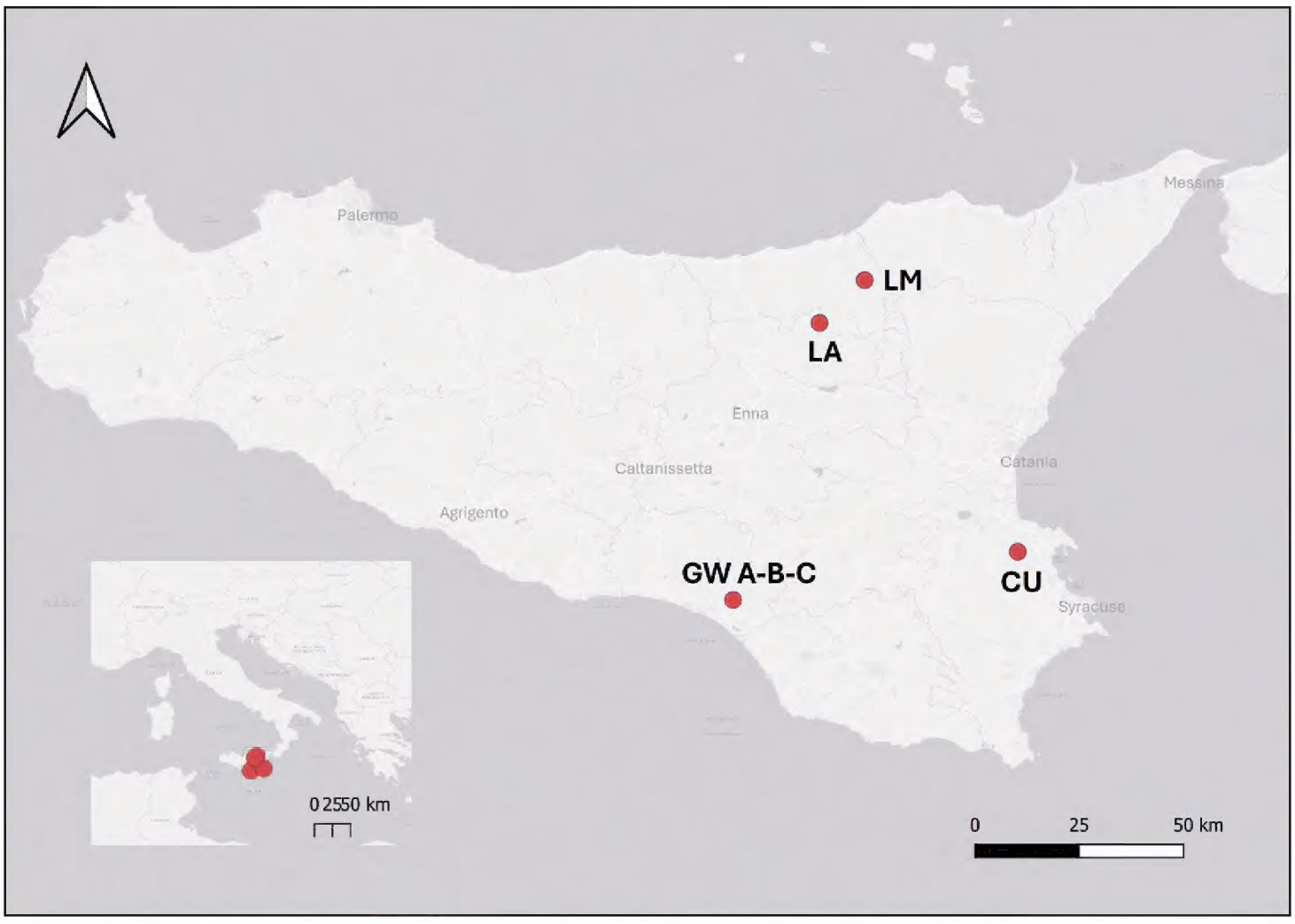

Figure 1. Map showing the sampling sites. Cesarò - Messina (LM), Gela - Caltanissetta (GW - A; GW - B; GW - C), Melilli - Syracuse (CU), and Troina - Enna (LA).

hosts many seasonal and permanent marshes, two artificial marshes were sampled; they were adjacent to each other and had brackish water because were formed by water from an underground saline spring. These marshes extend over a large area and salinity levels gradually decrease moving away from the spring. We sampled in the Acquitrino Principale del Giunco Foglioso (seasonal) and in the Pantano Pavoncelle (permanent); in the latter, we sampled in two different points, one more distant to the Acquitrino Principale del Giunco Foglioso but close to the opening of the saline aquifer, and one among the other two points (Fig. 2).

In Melilli sampling was carried out in the Curcuraggi area, an area mainly frequented by grazing cattle and characterised by the presence of temporary pools used as drinking troughs, in one of these we carried out the sampling. This temporary pool was located approximately 1 km from the Natura 2000 site ITA090024 at approximately 190 m asl.

Figure 2. Map showing the sampling sites in Geloi Wetland.

Table 1. Summary of information regarding the sampling sites.

Sampling sites	Abbreviation	Coordinates	Altitude
Curcuraggi - Melilli	CU	37°13'32.9"N, 15°05'38.0"E	about 190 m asl
Geloi Wetland - Gela	GW - A Acquitrino Principale del Giunco Foglioso	37°06'14.3"N, 14°20'18.5"E	about 30 m asl
	GW - B Pantano delle Pavoncelle (between the sampling point GW - A and GW - C)	37°06'12.9"N, 14°20'13.1"E	
	GW - C Pantano delle Pavoncelle (close to the opening of the saline aquifer)	37°06'13.9"N, 14°20'15.1"E	
Lago Ancipa - Sartori - Troina	LA	37°50'11.0"N, 14°33'53.5"E	about 947 m asl
Laghetto Monte Soro - Cesarò	LM	37°57'01.7"N, 14°41'07.0"E	about 1,575 m asl

Sampling and identification of Characeae

Samples of Characeae were collected by hand, placed in plastic bags containing the same water as the sampling sites, and put in a box with ice to carry them to the Phycology Laboratory of the Department of Biological, Geological and Environmental Sciences - University of Catania. Afterwards, the thalli were washed with distilled water to remove sediment and organic matter and, through a morphological and reproductive study by observation with stereoscopes and microscopes, following the dichotomous keys provided by Bazzichelli and Abdelahad (2009) and descriptions available from AlgaeBase (Guiry and Guiry 2024), they were identified. After that, the thalli were photographed using a digital camera Panasonic DC-FZ82 and, subsequently, the photos were processed using GIMP 2.10. The most significant specimens of the species found are held in CAT Sectio Algae Herbarium exiccata of the University of Catania.

Results

Examination of the samples revealed the presence of *Chara globularis* Thuiller in CU, *Chara vulgaris* Linnaeus in LA and GW-B, *Nitella capillaris* (Krocker) J.Groves & Bullock-Webster in LM, and *Tolypella glomerata* (Desvaux) Leonhardi in GW-A, GW-B and GW-C.

Details of the morphology, geographical distribution, conservation status, previous reports in Sicily and new reports in Sicily are provided below for the species recorded.

Chara globularis Thuiller

Fig. 3a–d

Geographical distribution. *C. globularis* is a cosmopolitan species (Urbaniak and Gabka 2014). It has been reported from United States (Smith 2010), Israel (Barinova and Romanov 2016), Iceland (Hrafnsdottir et al. 2019), and recently from Socotra Island, Yemen (Zalat et al. 2020) and India (Verma et al. 2021); it is also widespread in Europe (Guiry and Guiry 2024). In Italy, it has been reported from Trentino Alto Adige, Piedmont, Lombardy, Veneto, Friuli Venezia Giulia, Tuscany, Marche, Umbria, Latium, Sardinia and Sicily (Naselli-Flores and Barone 2002; Bazzichelli and Abdelahad 2009; Azzella 2014; Becker 2019; Guarino et al. 2019; Romanov et al. 2019; Panzeca et al. 2021).

Conservation status. In the Scandinavian countries, Czech Republic, Balkan countries, Switzerland and many German Länder *C. globularis* is classified as least concern (LC) while, in other regions of Germany (Saarland, Saxony and Thuringia) it is considered vulnerable (VU) or endangered (EN) (Auderset Joye and Rey-Boissezon 2013). In Hungary, this species is considered vulnerable (VU) by the International Union for Conservation of Nature and Natural Resources IUCN (Azzella 2014) and in Wales, it is in the least concern (LC) threat status of Red List Assessment (Stewart and

Figure 3. *Chara globularis* Thuiller **a** habit **b** rays with 6–7 cortical articles **c** small, rudimentary, papiliform stipulodes arranged in two rows **d** detail of some oogonia and antheridia. Scale bars: 1 cm (**a, b**); 100 μm (**c, d**).

Hatton-Ellis 2020). Finally in Slovakia *C. globularis* is a near-threatened (NT) species in IUCN red-list (Hindáková et al. 2022).

Previous reports for Sicily. Palermo, Pond of Santa Rosalia (Naselli-Flores and Barone 2002); Palermo, pond of Rebuttone (Guarino et al. 2019; Romanov et al. 2019); Palermo, Coda di Riccio lake (Guarino et al. 2019); Palermo, some ponds in the Palermo district (Panzeca et al. 2021).

New reports in Sicily. Curcuraggi (CU - Melilli, Syracuse).

Morphology. Thalli are 24–30 cm tall and green in colour. The main axis has a diameter of about 400 μm. The internodes are generally much longer than the branches (Fig. 3a). The rays are slender, with 6–7 corticate articles and two small terminal bare cells (Fig. 3b). The cortex is triplostichous, isostichous. It has rudimentary spine cells and the stipulodes are in two rows and are very small, rudimentary, and papilliform (Fig. 3c). The species is monoecious: the gametangia are borne jointly at the three lowest branchlet nodes; the oogonia are solitary and have a size of about 500 × 350 μm while the antheridia are about 300 μm in diameter (Fig. 3d).

***Chara vulgaris* Linnaeus**
Fig. 4a–d

Geographical distribution. *C. vulgaris* is a cosmopolitan species common in all continents (Korsch 2018). It occurs in Asia, America, and Europe (Guiry and Guiry 2024). In Italy, it has been reported from Trentino Alto Adige, Piedmont, Lombardy, Veneto, Liguria, Friuli Venezia Giulia, Emilia Romagna, Tuscany, Marche, Umbria, Abruzzo, Latium, Basilicata, Calabria, Apulia, Sardinia and Sicily (Bazzichelli and Abdelahad 2009; Troia and Barone 2017; Becker 2019; Guarino et al. 2019; Panzeca et al. 2021).

Conservation status. In Norway, *C. vulgaris* is classified as endangered (EN). In the Balkan countries, Sweden, and the Czech Republic it is considered at low risk of extinction, while in the German Länder, its status varies from vulnerable (VU) to least concern (LC). In Switzerland, it is vulnerable (VU) (Auderset Joye and Schwarzer 2012), in Wales in least concern (LC) threat status (Stewart and Hatton-Ellis 2020) and in Slovakia is a near-threatened (NT) species in IUCN red-list (Hindáková et al. 2022).

Previously reports from Sicily. Undefined locality in Sicily (Troia and Barone 2017); Palermo, Rocca dell'Aquila pond; Palermo, Rebuttone pond; South-east Sicily, Hyblaean Marshes; Trapani, Anguillara ponds; Palermo, portella Maganoce pond (Guarino et al. 2019); Palermo, some ponds in the Palermo district (Panzeca et al. 2021).

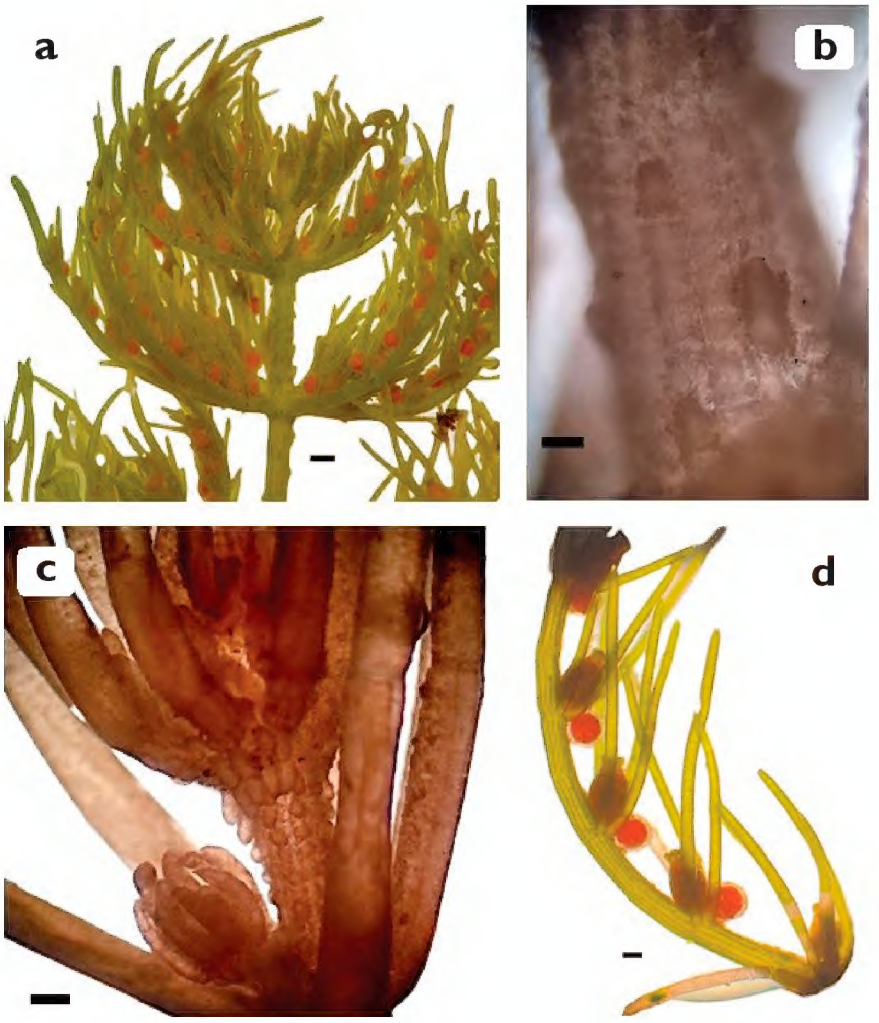

Figure 4. *Chara vulgaris* Linnaeus **a** habit of the apical part of an axis showing rays with the non-corticated terminal segments **b** diplostichous and aulacanthous cortex **c** detail of some spine cells and stipulodes arranged in two rows **d** detail of an axis with some oogonia and antheridia. Scale bars: 600 µm (**a**); 300 µm (**c**); 200 µm (**d**); 100 µm (**b**).

New reports in Sicily. Geloi Wetland (GW-B - Gela, Caltanissetta); Ancipa-Sartori lake (LA - Troina, Enna).

Morphology. Thalli are 10–30 cm tall. The main axis has a diameter of 600 µm, and the thalli are mainly green in colour but, sometimes, are also whitish/grey due to encrustations. The rays, arranged in whorls around the erect axes, are slightly curved upwards. Almost all segments are corticated, and the 2–3 terminal cells of the segments are consistently uncorticated (Fig. 4a). The cortex is diplostichous and aulacanthous (Fig. 4b). The spine cells are always solitary and papilliform. The stipulodes are clearly visible and arranged in two rows (Fig. 4c). The species is monoecious and bears both oogonia and antheridia on the same thalli (Fig. 4d). The oogonia are about 600 × 300 µm in size and the antheridia are about 350 µm in diameter.

Nitella capillaris (Krocker) J.Groves & Bullock-Webster

Fig. 5a–c

Geographical distribution. *N. capillaris* is not a widespread species. It has been mainly reported from Europe (Guiry and Guiry 2024) and Africa (Muller et al. 2017). In Italy, it was reported in Lombardy, Veneto, Emilia Romagna, Umbria, Latium, and Sicily (Bazzichelli and Abdelahad 2009; Troia et al. 2018; Guarino et al. 2019; Romanov et al. 2019).

Conservation status. In Sweden, Slovakia and Great Britain *N. capillaris* is considered extinct. In North Rhine-Westphalia, Schleswig-Holstein, Brandenburg and Thuringia is considered endangered and, following the revision of the Red List in 2010, in the Balkan countries the species is classified as vulnerable (VU) (Auderset Joye and Schwarzer 2012; Hindáková et al. 2022). In Poland, it is protected by national environmental laws and it is considered a critically endangered species (CR) by IUCN (Urbaniak and Gabka 2014).

Previous reports in Sicily. Catania, unspecified locality; Palermo, Gurgo di San Ciro (Formiggini 1908 reported as *Nitella capitata*); Trapani, Anguillara pond (Troia et al. 2018; Guarino et al. 2019; Romanov et al. 2019)

New reports in Sicily. Monte Soro Lake (LM - Cesarò, Messina).

Morphology. thalli are about 10–15 cm tall. The axes have a diameter of about 500 µm and the colour of the thalli is green or light green. The internodes are generally 2–3 times longer than the branches. In the whorls, there are generally 6 or more rays, branched once, with 2–3 second-order rays (Fig. 5a). The rays have pointed unicellular terminal segments (dactyls) (Fig. 5b). The rays of the lower whorls are usually sterile, while those of the upper whorls are fertile. *N. capillaris* is a dioecious species. The oogonia are borne in the bifurcations of the rays, generally grouped in pairs, and are about 300 × 280 µm in size, with prominent ridges (Fig. 5c). Antheridia were not observed in our specimens.

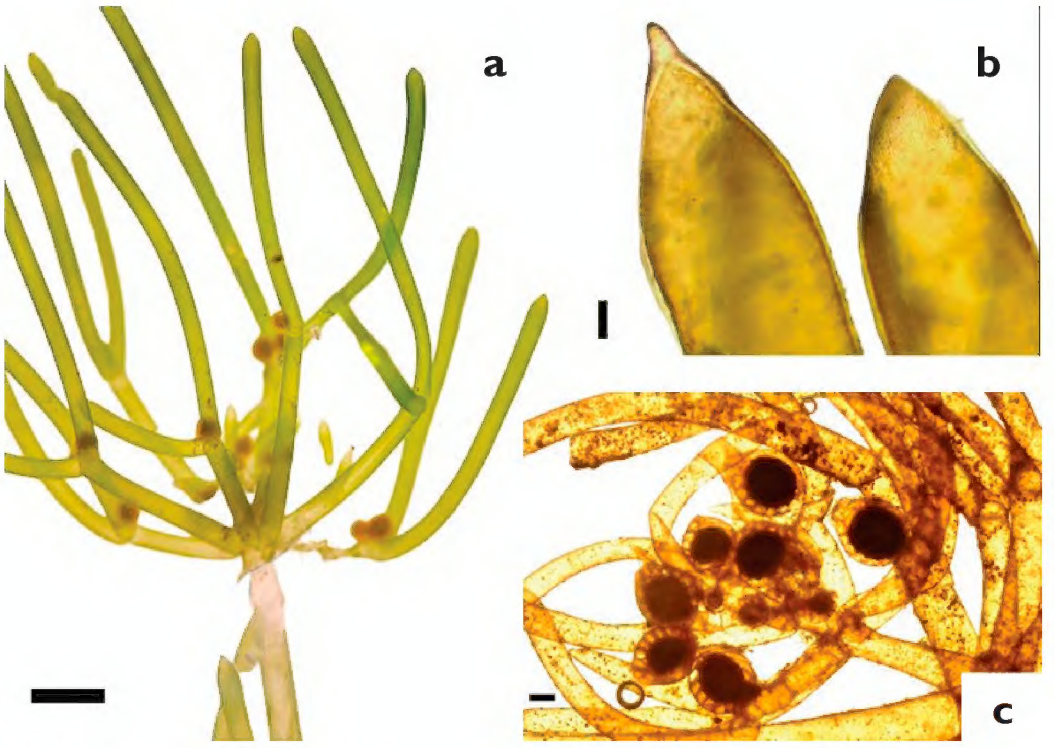

Figure 5. *Nitella capillaris* (Krocker) J.Groves & Bullock-Webster **a** rays branched once with the formation of 2–3 second-order rays **b** dactyl **c** detail of some oogonia. Scale bars: 400 µm (**a**); 100 µm (**b, c**).

Tolypella glomerata (Desvaux) Leonhardi

Fig. 6a–c

Geographical distribution. *T. glomerata* probably has a cosmopolitan distribution (Stewart and Hatton-Ellis 2020). It was recently reported from India (Verma et al. 2021) and Israel (Barinova and Smith 2022); from Europe in Iceland (Hrafnsdottir et al. 2019), Belgium (Denys et al. 2019), Germany (Täuscher 2020; Doege et al. 2022), and Britain (Pentecost and Haworth 2021) but, generally, it is widespread throughout the European continent (Guiry and Guiry 2024). In the British Isles its populations are widely distributed but scanty and apparently in regression (Stewart and Hatton-Ellis 2020). In Italy, it has been reported from Veneto, Emilia Romagna, Tuscany, Abruzzo, Basilicata, Apulia, Sardinia and Sicily (Formiggini 1908; Sciandrello 2007; Bazzichelli and Abdelahad 2009; Becker 2019).

Conservation status. In Wales this species is in the least concern (LC) threat status of Red List Assessment (Stewart and Hatton-Ellis 2020). In the Länder of Germany, the status of *T. glomerata* varies from extinct (RE) to vulnerable (VU), whereas in the Czech Republic it is considered endangered (CR). In Sweden, after the revision of the Red List in 2010, its status dropped by one category, and it is currently classified as critically endangered (EN) (Auderset Joye and Schwarzer 2012).

Previous reports in Sicily. Undefined location in Sicily (Formiggini 1908); Caltanisetta, Gela, Piana del Signore (Sciandrello 2007).

New reports in Sicily. Geloi Wetland (GW-A, GW-B, GW-C - Gela, Caltanissetta).

Morphology. Thalli are 7–10 cm tall, with erect axes about 1 cm thick; their colour is green with white encrustations. The branches are grouped in dense clusters

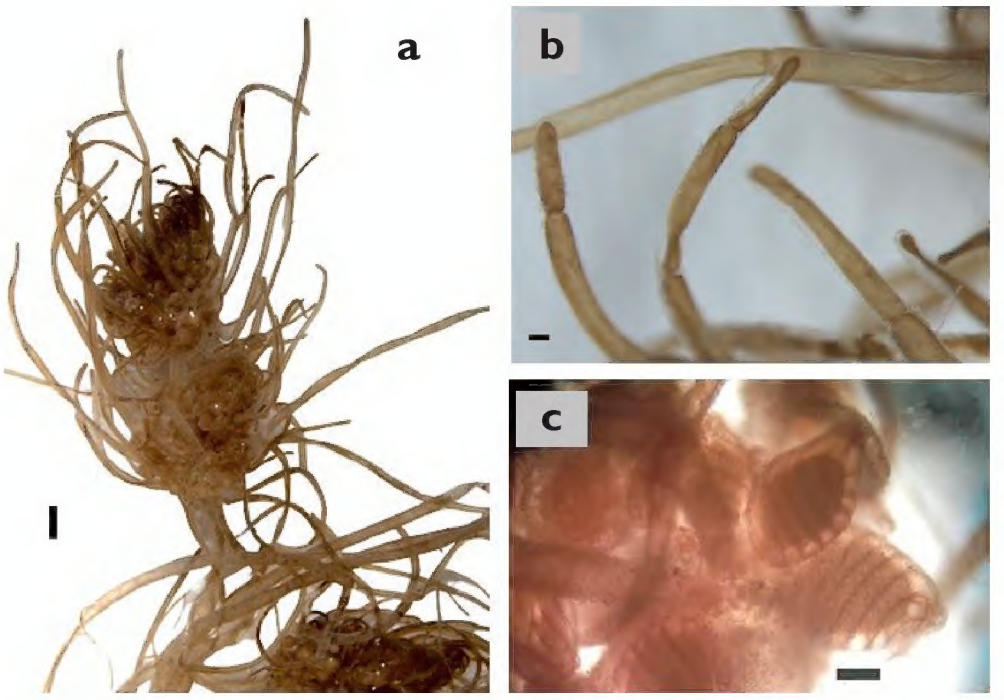

Figure 6. *Tolypella glomerata* (Desvaux) Leonhardi **a** branches agglomerated **b** segment of the rays with blunt apices **c** oogonia. Scale bars: 1 cm (**a**); 500 μm (**b**); 100 μm (**c**).

(Fig. 6a) and the whorls are composed of 6–8 rays each. The lower whorls are sterile and sparse, while the upper ones are fertile with dense rays. The terminal segments of the rays are shorter than the subterminal and their apices are blunt (Fig. 6b). *T. glomerata* is a monoecious species. The oogonia are borne in groups of 1–3 and are about 350 × 240 μm in size (Fig. 6c). The antheridia are about 250 μm in diameter.

Discussion

The results of this study improve the knowledge of the distribution of Characeae in eastern Sicily. None of the sampled species had been previously reported in the areas investigated and some scanty data were available only for nearby areas (Sciandrello 2007; Guarino et al. 2019; Romanov et al. 2019).

C. globularis has been reported from some wetlands in Palermo (Naselli-Flores and Barone 2002; Guarino et al. 2019; Romanov et al. 2019; Panzeca et al. 2021) but, as far as we know, there are no other reports for Sicily. Curcuraggi, where we found this species, unlike the other areas that we investigated, is impacted by human activities; it is indeed known that *C. globularis* can live in environments subject to anthropogenic impacts and that it is very tolerant to eutrophication (Auderset Joye and Schwarzer 2012; Barinova and Romanov 2016).

C. vulgaris is the most widespread and most reported species of *Chara* in Italy (Bazzichelli and Abdelahad 2009) and our records allow to add new sites, Geloi Wetland (GW-B) and Ancipa-Sartori lake (LA), which had previously not been inspected for the presence of Characeae.

N. capillaris was previously reported mainly in the areas of Trapani and Palermo and in 1908 in an undefined locality in Catania (Formiggini 1908; Guarino et al. 2019; Romanov et al. 2019). We found this species at Monte Soro Lake, located in the province of Messina

(LM), an area in which the species had not previously been reported. This lake is situated approximately at 1,575 m asl and often freezes in winter. This agrees with previous studies indicating that this species is able to survive during periods of frost, since it produces a large number of oospores that are resistant to freezing stress and germinate as soon as environmental conditions become favourable again (Auderset Joye and Schwarzer 2012).

Finally, we report the presence of *T. glomerata* in Geloi Wetland (Gela) (GW-A), (GW-B), (GW-C), south-eastern Sicily. This species had already been reported for the brackish marsh system of Piana del Signore (Gela) (Sciandrello 2007), very close to our site of discovery, but had not yet been reported for the wetland examined in the present study. As reported by Bazzichelli and Abdelahad (2009), Cirujano Bracamonte et al. (2013), and Zeneli and Kashta (2016), this species is often found in slightly brackish waters; accordingly, we found this species in the only site with this type of water. We noted that the population density and its fertility decreased moving from the Acquitrino del Giunco Foglioso (sal 0.09%, Ph 8.46) to the Pantano Pavoncelle (sal 0.13%, Ph 8.48), in which the salinity is higher due to a saltwater spring. This is in agreement with Winter et al. (1996), who reported that, despite being a species that adapts well to brackish environments, salinity is a limiting factor for the fertility of this species.

Conclusions

The results of this study show that the distribution of Characeae in Sicily is not yet sufficiently known, and more attention should be given to the presence of these interesting algae in Sicilian wetlands. This would be important to fill the knowledge gaps that still exist and to create, even in Italy, a national Red List for the protection of these species and the environments in which they live.

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